Search for Antagonistic Substances of Serotonin and Alpha Adrenergic Receptors from Natural Resources and Their Pharmacological Studies(セロトニン及びα-受容体拮抗作用を有する天然物の探索並びにそれらの薬理学的研究)

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論 文 内 容 要 旨

Plants have been employed in traditional medicinal practice as essential drugs for the treatment of various kinds of diseases for many centuries. However, with rapid progress in modern science and vast growth of pharmaceutical industries, plant crude drugs have been gradually replaced by chemical drugs whose quality and efficacy are much more reliable. Many clinically useful drugs and chemical as a pharmacological tool have been isolated from the crude drugs.

In Chinese (Kampo) medicine, the dried hooks and stems of *Uncaria* plants (Rubiaceae) have been used as a spasmolytic, an analgesic and sedative treatment of many symptoms associated with hypertension and cerebrovascular disorders. Many alkaloids have been isolated from these plants and shown to possess various pharmacological activities. It has been reported that geissoschizine methyl ether (Fig. 1A), an indole alkaloid isolated from the water extracts of the hooks of *U. sinensis* (Oliv.) Havil., inhibits the glutamate-induced convulsion. On the other hand, the fruit of *Nandina domestica* Thunberg (Berberidaceae) has been used in the treatment for asthma, whooping cough, pharynx tumor and uterine bleeding in Japan for many years. Chemical studies on the constituents of this plant have revealed the presence of numerous alkaloids. Initially, it was found that the crude methanol extract of the fruit selectively inhibited 5-HT- induced contractions of isolated rabbit aorta without affecting contractions induced by KCl or histamine. (+)-Nantenine(Fig.1 B) has been isolated as an antiserotonergic substance from this plant.

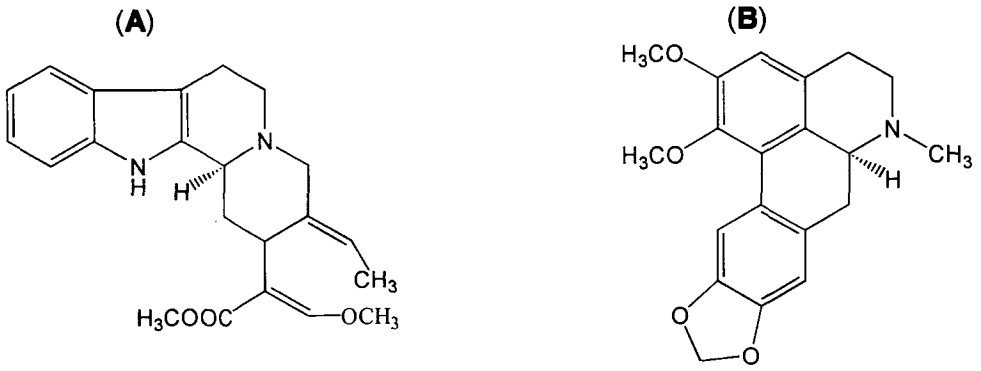


Fig. 1. Structure of geissoschizine methyl ether (A) and (+)-nantenine (B)

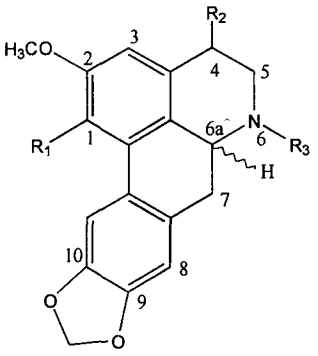
The medicinal usage and the preliminary pharmacological studies of geissoschizine methyl ether (GM) and (+)-nantenine have stimulated me to study and establish more detailed the pharmacological actions of these compounds. The purpose of this study is to discover new leading compounds for valuable drugs possessing antiserotonergic or antiadrenergic activity.

The present study, includes the pharmacological analysis of GM and (+)-nantenine in the central nervous system, structure-activity relationship (SAR) analysis on (±)-nantenine derivatives (Table 1) in antiserotonergic or antiadrenergic activity and evaluation of pharmacological properties of the most active compound from SAR analysis

by using rat and human alpha-1 adrenoreceptor (α_1-AR) subtypes.

Table 1 (±)-Nantenine derivatives

Compounds	R_1	R_2	R_3
(±)-Nantenine	OCH_3	H	CH_3
(±)-Nornantenine	OCH_3	H	H
(±)-Ethylnornantenine	OCH_3	H	C_2H_5
(±)-*N*-Trifluoroacetylnornantenine	OCH_3	H	$COCF_3$
(±)-4α-Hydroxynantenine	OCH_3	α-OH	CH_3
(±)-4β-Hydroxynantenine	OCH_3	β-OH	CH_3
(±)-Domesticine	OH	H	CH_3
(±)-Nordomesticine	OH	H	H
(±)-*N*-Trifluoroacetyldomesticine	OH	H	$COCF_3$



The pharmacological study of GM and (+)-nantenine in the central nervous system showed that GM and (+)-nantenine reduced the l-5-HTP plus clorgyline-induced head twitch response in a dose-dependent manner. The magnitude of head twitch response in the presence of GM and (+)-nantenine was significantly less than the response in vehicle-treated control. In binding experiments, GM and (+)-nantenine had a less affinity toward α_1-adrenoceptor (α_1-AR) or dopamine D_2 receptor than 5-HT_{1A}/ 5-$HT_{2A/2C}$ receptors in the mouse. However, l-5-HTP-plus clorgyline or 8-OH-DPAT-induced head weaving was not affected by both of the compound. These results may indicate that these compounds did not possess as 5-HT_{1A} antagonist activities. It has been reported that s.c. administration of 8-OH-DPAT (in doses up to 10 mg/kg) elicited hypothermia in mice but had no apparent behavioural syndrome. In the present study the i.p. administration of GM and (+)-nantenine produced hypothermic response. These results suggest that GM and (+)-nantenine possess mixed 5-HT_{1A} receptor agonist/5-$HT_{2A/2C}$ receptor antagonist activities and inhibits the head twitch response by blocking the 5-HT_{2A} receptors, and possibly, at least in part, by stimulating the 5-HT_{1A} receptors in the central nervous system.

The pharmacological profile of aporphine may change due to small structural changes. Therefore (±)-nantenine and its derivatives were synthesized to study the structure-activity relationship on the 5-HT_{2A} receptors and α_{1D}-AR as well as their pharmacological profile (Table 1). (±)-Nantenine and its derivatives produced competitive antagonistic activity not only in the 5-HT_{2A} receptor but also in α_{1D}-AR of rat aorta. The structure-activity relationship study showed that replacing of a methyl group at N-6 of (±)-nantenine with a hydrogen atom or an ethyl group decreased the affinity to the 5-HT_{2A} receptor and α_1-adrenoceptor. These data indicate that a methyl groups at N-6 is a crucial element in the interaction process between these alkaloids and their receptors. Furthermore, introducing a hydroxyl group at C-4 α β in the (+)-nantenine structure also reduced the affinity. Interestingly, displacement of a methoxy moiety at C-1 with a hydroxyl group caused a marked decrease in the affinity for the 5-HT_{2A} receptors but remained a high affinity for an α_1-adrenoceptor. Among (±)-nantenine derivatives (±)-domesticine is most powerful in α_1-adrenoceptor blocking action in rat aorta. The study on the structure-activity relationship has clarified the contribution of a hydroxyl group at C-1 position and a methyl group at

N-6 position in the aporphine skeleton of (±)-nantenine is essential to the development for the α_1 -adrenoceptor blocking activity.

Furthermore, the potency and selectivity of (±)-domesticine were examined on α_1 -AR subtypes using animal tissues, and Chinese hamster ovary (CHO) cells expressing cloned human α_1 -AR subtypes and compared with BMY 7378, a prototype selective α_{1D}-AR. The selectivity of (±)-domesticine to inhibit PE-induced contraction in rat thoracic aorta was 32- and 17-fold higher than that in tail artery and spleen, respectively, while degree of selectivity of BMY 7378 was 125- and 11-fold. The affinity profiles of these compounds for the α_1 -AR subtypes in functional experiments of animal tissues were consistent with respective

Table 2 Receptor subtypes selectivity of (±)-domesticine and BMY 7378

Tissue/cell	Receptor Subtype Selectivity	Compound	
		(±)-Domesticine	BMY 7378
Animal Tissues	α_{1A}/α_{1D}	32	125
	α_{1B}/α_{1D}	17	11
	5-HT_{1A}/α_{1D}	183	0.83
	*5-HT_{2A}/α_{1D}	72	154
	**5-HT_{2A}/α_{1D}	95	193
	5-HT_{2C}/α_{1D}	295	73
Human α_1–ARs	α_{1a}/α_{1d}	34	102
	α_{1b}/α_{1d}	9	21

binding affinity profiles in cloned human α_1 -AR subtypes (Table 2). (±)-Domesticine displayed a 34- and 9-fold higher selectivity for α_{1d}-AR than for α_{1a}-and α_{1b}-AR, respectively, while BMY 7378 showed a selectivity for α_{1d}-AR of 102-fold that of α_{1a}-AR and 21-fold that of α_{1b}-AR. Interestingly, in [^{3}H]8-OH-DPAT binding to 5-HT_{1A} receptors of rat cerebral cortex, (±)-domesticine showed a 183-fold higher selectivity for α_{1D}-AR relative to 5-HT_{1A} receptor, whereas BMY 7378 displayed a similar affinity at this receptor with respect to the α_{1D}-AR (0.89-fold). Both compounds, however, showed a weak affinity for 5-HT_{2A}/5-HT_{2C} receptors in rat frontal cortex. These results suggest that (±)-domesticine is more potent for α_{1D}-AR than for α_{1A}- or α_{1B}-AR subtypes and it is highly selective compared to 5-HT_{1A} and other receptors.

In conclusion, we found that both GM and (+)-nantenine are useful pharmacological tools for evaluating the functional role of the 5-HT receptor in central nervous system. A hydroxyl group at C-1 position and a methyl group at N-6 position of (±)-nantenine skeleton is essential to the development for the α_1 -adrenoceptor blocking activity. (±)-Domesticine is a selective α_{1D}-AR antagonist and may be promising for development of new drugs.

審査結果の要旨

人類は様々な病気の治療に，薬用植物を何世紀もの間用いてきた。近代科学特に薬学の急速な発展に伴い，これらの植物は次第により品質と効力の信頼が置ける合成薬に次第にとって代わられつつある。しかしながら，臨床応用される薬や薬理学的ツールとして用いられる多くの化合物は天然由来のものである。

まず，インドラ氏は抗セロトニンあるいは抗アドレナリン作用を持つ有用な薬物開発のための新しいリード化合物を見つけるため，*Uncaria sinensis* (Oliv.) Havil. から単離された geissoschizine methyl ether (GM) および *Nandina domestica* Thunberg の果実の成分(+)-nantenine の薬理作用を詳細に明らかにすることから研究を開始した。

GM および(+)-nantenine は，*l*-5-HTP と clorgyline によって誘発される head twitch 反応を濃度依存性に抑制することを見出した。両化合物は5-HT_{1A}/5-$HT_{2A/2C}$受容体に比べてα_1アドレナリン受容体及びドパミン受容体に対する親和性が低いこと，また5-HT_{2A}受容体阻害と，おそらく少なくとも部分的には，5-HT_{1A}受容体の活性化によって head twitch 反応を抑制することが明らかとなった。

次に，(±)-nantenine とその誘導体を合成し，5-HT_{2A}受容体およびα_{1D}受容体における構造活性相関を検討した結果，(±)-nantenine のN-6のメチル基がこれらのアルカロイドと受容体の相互作用に必須の因子であることが明らかとなった。また，aporphine 骨格のC-1の水酸基がα_1受容体阻害作用発現に必須であることが明らかとなった。

さらに，動物組織ならびにヒトα_1受容体サブタイプを発現させたチャイニーズ・ハムスターの卵(CHO)細胞を用いて，(±)-domesticine のα_1アドレナリン受容体サブタイプに対する効力と選択性を検討した。その結果，(±)-domesticineのα_{1d}受容体に対する選択性はα_{1a}およびα_{1b}受容体に比してそれぞれ34倍と9倍高いこと，さらに興味深いことに，ラット大脳皮質上の5-HT_{1A}受容体に対する [^{3}H]8-OH-DPAT の結合性と比べると，(±)-domesticineのα_{1D}受容体の選択性は180倍高いことが明らかとなった。これらの結果から，(±)-domesticineはα_{1A}やα_{1B}受容体に比べてα_{1D}受容体への強い選択性があることが示された。

以上の結果から，中枢神経系における5-HT 受容体機能の評価の薬理学的ツールとして GMと(+)-nantenine が有用であること，また，(±)-domesticine がα_{1D}受容体の選択的拮抗阻害薬であり，新しいα受容体遮断薬のリード化合物としてその開発研究の進歩に貢献すると期待される。

よって，本論文は博士（薬学）の学位論文として合格と認める。